

Preliminary Amendment

Page 7

Applicant(s): Salman Baig et al.

Serial No. Unknown (National Stage of PCT/US00/10672)

Filed: Herewith (Int'l. Filing Date: April 20, 2000)

For: CYSTEINE PROTEASE AND INHIBITORS FOR PREVENTION AND TREATMENT OF NEUROCYSTICERCOSIS

Remarks

Claims 4-6, 8-10, 14, 15, 17-25, 28, 29, 31-34, 39, 42 and 43 having been amended and claims 44-48 have been added, the pending claims are 1-48.

Amendments to the claims have been made to remove multiple dependency, and to clarify the claimed subject matter.

Conclusion

The Examiner is invited to contact Applicants' Representatives at the below-listed telephone number, if there are any questions regarding this Preliminary Amendment or if prosecution of this application may be assisted thereby.

Respectfully submitted for

Salman Baig et al.

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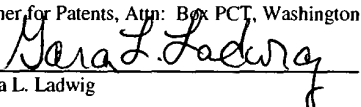
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APPENDIX A - SPECIFICATION/CLAIM AMENDMENTS
INCLUDING NOTATIONS TO INDICATE CHANGES MADE
Serial No.: Unknown (National Stage of PCT/US00/10672)
Docket No. 235.0027 0101

Amendments to the following are indicated by underlining what has been added and bracketing what has been deleted. Additionally, all amendments have been shaded.

In The Title

The title has been amended as follows:

CYSTEINE PROTEASE AND INHIBITORS FOR PREVENTION AND
TREATMENT OF NEUROCYSTICERCOSIS [NEUROCYSTICEROCOSIS]

In the Claims

For convenience, all pending claims are shown below.

1. An isolated cyst wall cysteine proteinase derived from *Taenia solium*.
2. An isolated polynucleotide having a nucleic acid sequence that encodes the proteinase of claim 1.
3. An isolated polynucleotide that is the complement of the nucleic acid molecule of claim 2.
4. (AMENDED) A vaccine comprising a cyst wall cysteine proteinase or a polynucleotide having a nucleic acid sequence encoding a cyst wall cysteine proteinase, and a pharmaceutically acceptable carrier, for use as a vaccine].
5. (AMENDED) A method for preparing the vaccine of claim 4 comprising mixing [Use of] a cyst wall cysteine proteinase or a polynucleotide having a nucleic acid sequence encoding a cyst wall cysteine proteinase, and a pharmaceutically acceptable carrier [for the preparation of a vaccine].

Appendix A

Page A-2

Applicant(s): Salman Baig et al.

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For: CYSTEINE PROTEASE AND INHIBITORS FOR PREVENTION AND TREATMENT OF
NEUROCYSTICERCOSIS

6. (AMENDED) A vaccine comprising at least one component selected from the group consisting of (a) a polypeptide comprising a cyst wall cysteine proteinase or an immunogenic polypeptide subunit thereof and (b) a polynucleotide comprising a nucleotide sequence encoding a polypeptide comprising a cyst wall cysteine proteinase or an immunogenic polypeptide subunit thereof and a pharmaceutically acceptable carrier.

7. The vaccine of claim 6 wherein the cyst wall cysteine proteinase is derived from *T. solium* or *T. crassiceps*.

8. (AMENDED) A method for treating an animal harboring a *Taenia* infection comprising administering to the infected animal the vaccine of claim 6 [claims 6 or 7], wherein administration of the vaccine is effective to eliminate the parasite from the animal or to prevent or delay the appearance of cysticercosis or neurocysticercosis the animal.

9. (AMENDED) A method for protecting an animal against a *Taenia* infection comprising administering to an uninfected animal the vaccine of claim 6 [claims 6 or 7], wherein administration of the composition is effective to prevent subsequent infection of the animal by the parasite or to prevent the development of cysticercosis or neurocysticercosis in the animal after subsequent infection by the parasite.

10. (AMENDED) The method of claim 8 [claims 8 or 9] wherein the animal is a pig or a human and the *Taenia* infection is a *T. solium* infection.

11. A pharmaceutical composition comprising:

an inhibitor molecule that inhibits the activity of a cyst wall cysteine protease, the inhibitor molecule comprising a peptide or peptidomimetic compound; and
a pharmaceutically acceptable carrier.

Appendix A

Page A-3

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For: CYSTEINE PROTEASE AND INHIBITORS FOR PREVENTION AND TREATMENT OF
NEUROCYSTICERCOSIS

12. The pharmaceutical composition of claim 11 wherein the peptide or peptidomimetic compound comprises (Xaa)_n-Yaa-Zaa-R; wherein Xaa and Zaa are each independently any amino acid; Yaa is a hydrophobic amino acid; R comprises a nucleophilic moiety; and n = 0-5.

13. The pharmaceutical composition of claim 12 wherein Yaa is leucine.

14. (AMENDED) The pharmaceutical composition of **claim 12 [claims 12 or 13]** wherein R comprises a chemical moiety selected from the group consisting of a carboxylic acid derivative, an amide derivative, a benzene derivative, a phenyl derivative, a chloromethylketone or derivative thereof, a fluoromethylketone or derivative thereof, an alphaketo acid or derivative thereof, a ketoamide or derivative thereof, a ketoester or derivative thereof, a vinylsulfone or derivative thereof, and a pyridyl or derivative thereof.

15. (AMENDED) The pharmaceutical composition of **claim 12 [claims 12-14]** wherein R comprises a fluoromethylketone (FMK).

16. The pharmaceutical composition of claim 15 wherein the inhibitor molecule is Z-Leu-Leu-Leu-FMK or Leu-Leu-Tyr-FMK.

17. (AMENDED) The pharmaceutical composition of claim 11 **[16]** wherein the inhibitor molecule inhibits cyst wall cysteine proteinase derived from *T. solium* or *T. crassiceps*.

18. (AMENDED) **A pharmaceutical composition comprising** Z-Leu-Leu-Leu-FMK or Z-Leu-Leu-Tyr-FMK; **and a pharmaceutically acceptable carrier [for use as an active pharmaceutical substance].**

Appendix A

Page A-4

Applicant(s): Salman Baig et al.

Serial No. Unknown (National Stage of PCT/US00/10672)

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For: CYSTEINE PROTEASE AND INHIBITORS FOR PREVENTION AND TREATMENT OF NEUROCYSTICERCOSIS

19. (AMENDED) A method for [Z-Leu-Leu-Leu-FMK or Z-Leu-Leu-Tyr-FMK according to claim 18 for use in] treating human neurocysticercosis comprising administering to a human subject the pharmaceutical composition of claim 18 [or porcine cysticercosis].

20. (AMENDED) A method for treating porcine cysticercosis comprising administering to a porcine subject the [The use of Z-Leu-Leu-Leu-FMK or Z-Leu-Leu-Tyr-FMK for the preparation of a] pharmaceutical composition of claim 18.

21. (AMENDED) A method for treating human neurocysticercosis or porcine cysticercosis comprising administering to a human or porcine subject a pharmaceutical composition comprising [The use of] an inhibitor molecule that inhibits the activity of a *Taenia* cysteine protease, the inhibitor molecule comprising a peptide or peptidomimetic compound, and a pharmaceutically acceptable carrier[, for the treatment of human neurocysticercosis or porcine cysticercosis].

22. (AMENDED) The method of [use of an inhibitor molecule according to] claim 21 wherein the peptide or peptidomimetic compound comprises (Xaa)_n-Yaa-Zaa-R; wherein Xaa and Zaa are each independently any amino acid; Yaa is a hydrophobic amino acid; R comprises a nucleophilic moiety; and n = 0-5.

23. (AMENDED) The method of [use of an inhibitor molecule according to] claim 22 wherein Yaa is leucine.

24. (AMENDED) The method of claim 22 [use of an inhibitor molecule according to claims 22 or 23] wherein R comprises a chemical moiety selected from the group consisting of a carboxylic acid derivative, an amide derivative, a benzene derivative, a phenyl derivative, a

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chloromethylketone or derivative thereof, a fluoromethylketone or derivative thereof, an alphaketo acid or derivative thereof, a ketoamide or derivative thereof, a ketoester or derivative thereof, a vinylsulfone or derivative thereof, and a pyridyl or derivative thereof.

25. (AMENDED) The method of claim 22 use of an inhibitor molecule according to claims 22-24 wherein R comprises a fluoromethylketone (FMK).

26. A method for inhibiting *Taenia* cyst wall cysteine protease activity comprising contacting a *Taenia* cyst wall cysteine protease with an inhibitor molecule comprising (Xaa)_n-Yaa-Zaa-R; wherein Xaa and Zaa are each any amino acid; Yaa is a hydrophobic amino acid; R comprises a nucleophilic moiety; and n is 0 to about 5.

27. The method of claim 26 wherein Yaa is leucine.

28. (AMENDED) The method of claim 26 claims 26 or 27 wherein R comprises a chemical moiety selected from the group consisting of a carboxylic acid derivative, an amide derivative, a benzene derivative, a phenyl derivative, a chloromethylketone or derivative thereof, a fluoromethylketone or derivative thereof, an alphaketo acid or derivative thereof, a ketoamide or derivative thereof, a ketoester or derivative thereof, a vinylsulfone or derivative thereof, and a pyridyl or derivative thereof.

29. (AMENDED) The method of claim 26 -28 wherein R comprises a fluoromethylketone (FMK).

30. The method of claim 29 wherein the inhibitor molecule is Z-Leu-Leu-Leu-FMK or Z-Leu-Leu-Tyr-FMK.

Appendix A

Page A-6

Applicant(s): Salman Baig et al.

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For: CYSTEINE PROTEASE AND INHIBITORS FOR PREVENTION AND TREATMENT OF
NEUROCYSTICERCOSIS

31. (AMENDED) The method of claim 26 [30] performed in cell free environment.

32. (AMENDED) The method of claim 26 [30] performed in cell culture, in an organ, or in a tissue.

33. (AMENDED) The method of claim 26 [30] performed in a whole animal.

34. (AMENDED) The method of claim 26 [30] wherein the *Taenia* cysteine proteinase is derived from *T. solium*.

35. A method for identifying an inhibitor of *Taenia* cysteine proteinase activity comprising:
combining a candidate inhibitor and a *Taenia* cysteine proteinase to form a mixture;
adding the proteinase substrate Z-Phe-Arg-7-amino-4-trifluoromethylcoumarin to the mixture; and

determining the extent to which the proteinase substrate is cleaved;
wherein a reduction in the extent of substrate cleavage compared to the extent of substrate cleavage in the absence of the candidate inhibitor is indicative of inhibition of *Taenia* cysteine proteinase activity.

36. The method of claim 35 comprising combining a candidate inhibitor and a cysteine protease derived from *T. solium* or *T. crassiceps* to form the mixture.

37. A computer-assisted method for identifying an inhibitor of *Taenia* cysteine proteinase activity comprising:

supplying a computer model of the structure of an inhibitor of *Taenia* cysteine proteinase activity;

Appendix A

Page A-7

Applicant(s): Salman Baig et al.

Serial No. Unknown (National Stage of PCT/US00/10672)

Filed: Herewith (Int'l. Filing Date: April 20, 2000)

For: CYSTEINE PROTEASE AND INHIBITORS FOR PREVENTION AND TREATMENT OF NEUROCYSTICERCOSIS

either (i) computationally or visually screening a structural library for candidate compounds having a structure similar to that of the inhibitor or (ii) computationally or visually designing a candidate compound having a structure similar to that of the inhibitor; and assaying the candidate compound for the ability to inhibit *Taenia* cysteine proteinase activity.

38. A computer-assisted method for identifying an inhibitor of *Taenia* cysteine proteinase activity comprising:

solving the X-ray crystal structure of a *Taenia* cysteine proteinase to yield a computer model of the *Taenia* cysteine proteinase;

supplying a computer model of the structure of an inhibitor of *Taenia* cysteine proteinase activity;

computationally or visually docking the inhibitor structure to the proteinase crystal structure at the binding site of the inhibitor;

computationally or visually identifying intermolecular interactions between the inhibitor and the proteinase;

either (i) computationally or visually screening a structural library for candidate compounds having a structure similar to that of the inhibitor or (ii) computationally or visually designing a candidate compound having a structure similar to that of the inhibitor;

computationally or visually evaluating the intermolecular interactions between the candidate compound and the proteinase; and

assaying the candidate compound for the ability to inhibit *Taenia* cysteine proteinase activity.

39. (AMENDED) The method of **claim 37 [claims 37 or 38]** wherein the inhibitor is Z-Leu-Leu-Leu-FMK or Z-Leu-Leu-Tyr-FMK.

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NEUROCYSTICERCOSIS

40. A method for treating an animal harboring a *Taenia* infection comprising administering to the infected animal a pharmaceutical composition comprising an inhibitor of a *Taenia* cysteine proteinase, wherein administration of the composition is effective to eliminate the parasite from the animal or to prevent or delay the appearance of cysticercosis or neurocysticercosis the animal.

41. A method for protecting an animal against a *Taenia* infection comprising administering to an uninfected animal a pharmaceutical composition comprising an inhibitor of a *Taenia* cysteine proteinase, wherein administration of the composition is effective to prevent subsequent infection of the animal by the parasite or to prevent the development of cysticercosis or neurocysticercosis the animal after subsequent infection by the parasite.

42. (AMENDED) The method of **claim 40 [claims 40 or 41]** wherein inhibitor is Z-Leu-Leu-Leu-FMK or Z-Leu-Leu- Tyr-FMK.

43. (AMENDED) The method of **claim 40 [claims 40, 41 or 42]** wherein the animal is a pig or a human and the *Taenia* infection is a *T. solium* infection.

44. (NEW) The method of claim 9 wherein the animal is a pig or a human and the *Taenia* infection is a *T. solium* infection.

45. (NEW) The method of claim 38 wherein the inhibitor is Z-Leu-Leu-Leu-FMK or Z-Leu-Leu-Tyr-FMK.

46. (NEW) The method of claim 41 wherein inhibitor is Z-Leu-Leu-Leu-FMK or Z-Leu-Leu-Tyr-FMK.

Appendix A

Page A-9

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For: CYSTEINE PROTEASE AND INHIBITORS FOR PREVENTION AND TREATMENT OF
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47. (NEW) The method of claim 41 wherein the animal is a pig or a human and the *Taenia* infection is a *T. solium* infection.

48. (NEW) The method of claim 42 wherein the animal is a pig or a human and the *Taenia* infection is a *T. solium* infection.